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Effect of Vegetable Oils on Plasma Lipids of Rhesus Monkeys.* (27188)

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The effect of dietary fats on serum cholesterol has been the subject of many recent investigations. Ahrens(1) reviewed the contributions of different workers, including his own group, which showed that the concentration of serum cholesterol in man is influenced by the nature of the dietary fat. Connor *et al.*(2) recently reported that dietary cholesterol is one of the important factors which influence the blood cholesterol level in human beings. In experiments with rats Swell and Flick(3) observed that oleic acid feeding resulted in higher blood cholesterol levels than did stearic acid. Lin *et al.*(4,5) also observed increased fecal excretion of cholesterol when the animals were fed palmitic acid or stearic acid. Aftergood *et al.*(6) observed that diets based on lard resulted in higher serum cholesterol than did diets based on cottonseed oil. Portman *et al.*(7) found in Cebus monkeys, that those fats which were most rich in polyunsaturated fatty acids tended to promote the lowest serum choleserol level. Sunflowerseed oil, an unsaturated fat, lowers serum cholesterol while hydrogenated coconut oil produces a sustained rise of serum cholesterol (8). These studies indicated a possible inverse relationship between iodine number of dietary fat and serum cholesterol. Jones *et al.*(9) observed, in cholesterol fed chicks, a cholesterol depressing effect of corn oil, but showed that this effect could not be explained by the fatty acid component of the oil. Experimental data on other animals including man also did not prove the hypothesis that degree of unsaturation and essential fatty acid composition of dietary fat controlled the serum cholesterol level(4,10,11). The differences between animal species complicate the picture. More information is needed regarding the properties of dietary fat and the effect of their prolonged administration on serum cholesterol level.

The present work deals with the effects of feeding 3 edible oils commonly used in India, mustard oil, coconut oil and sesame oil, on the different fractions of plasma lipids in rhesus monkeys.

Materials and methods. Eleven rhesus monkeys were fed a basal diet consisting of wheat flour 64 parts, ground Bengal gram (*Cicer arietinum*) 20 parts, commercial casein 12 parts, calcium carbonate 3 parts and common salt 1 part. The diet contained 2% fat, 19% protein, 65% carbohydrate and 1% fiber. Cholesterol of the diet was extracted with petroleum ether in a soxhlet apparatus, petroleum ether removed, the residue extracted with acetone, alcohol was added, hydrolysed with a drop of 33% potassium hydroxide, cholesterol precipitated with digitonin and estimated by the method of Sobel and Meyer(12). Cholesterol of the diet was 15 mg per 100 g possibly derived from crude casein. The animals were fed in addition 50 mg ascorbic acid per animal per day and 5 drops of a concentrate of Vit. A and D twice a week. The animals grew normally and consumed 150 g of the diet per day. Average weight of the animals was 4 kg.

Blood was collected by vein-puncture on the morning after an over night fast, for analysis of the different fractions of plasma lipids. The animals were then divided into 5 groups and were fed basal diet only, basal diet with 3 g cholesterol mixed with the diet,

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3 g cholesterol and 15 g mustard oil mixed with the basal diet, 3 g cholesterol and 15 g coconut oil mixed with the basal diet, and 3 g cholesterol and 15 g sesame oil mixed with the basal diet. Isocaloric diet was given to animals of the different groups. The iodine values of the oils were determined by the iodine monochloride method as described in British Pharmacopeia (25). The iodine values of the different oils were as follows: Mustard oil—104, coconut oil—9 and sesame oil—110. Feces were collected daily, dried and preserved for analysis of total cholesterol. Feces were extracted with petroleum ether in a soxhlet apparatus, petroleum ether was removed from an aliquot of the measured extract, residue dissolved in acetone, alcohol added, hydrolysed with a drop of 33% potassium hydroxide, treated with digitonin, the precipitate of cholesterol digitonide was analysed for cholesterol by the method of Sobel and Meyer(12). Fasting blood samples were collected at intervals after feeding the different diets and analysed for free and ester cholesterol(12), phospholipids (13), nonesterified fatty acids(14) and lipoproteins(15). Plasma was pre-stained with acetylated Sudan Black B before electrophoresis on paper and the values were expresed as α-lipoprotein and β + O lipoprotein. The results are given in Tables I-V. Fatty acid composition of the oils is given in Table VI.

Results. The feeding of cholesterol for 4 months did not affect the plasma total and ester cholesterol. A slight elevation in cholesterol level was observed after cholesterol was fed for 6 months. Plasma total cholesterol began to increase from the first month of feeding of cholesterol when the diet was supplemented with mustard oil, coconut oil and sesame oil but the rise was maximum in

TABLE I. Total Plasma Cholesterol of Monkeys on Different Diets (mg/100 ml).

Monkey No.	Treatment	Months after supplements were fed 0	½	1	2	3	4	6
8	Basal diet only	90		91	90	91		—
10	*Idem*	122		119	120	118		123
11	"	101		101	106	108		118
3	Basal + cholesterol, 3 g/day	88	89	89	86	85	84	108
5	" " "	110	110	110	110	106	104	121
6	*Idem* + mustard oil, 15 g/day	117	118	136	172	193	200	241
7	" " "	104	110	135	162	178	200	241
1	" + coconut oil, "	91	92	135	176	213	222	231
4	" " "	98	102	130	152	162	173	193
13	" + sesame oil, "	131	131	174	295	380	510	736
14	" " "	118	117	148	199	251	310	644

TABLE II. Plasma Ester Cholesterol of Monkeys on Different Diets (mg/100 ml).

Monkey No.	Treatment	Months after supplements were fed 0	½	1	2	3	4	6
8	Basal diet only	59		61	58	59		—
10	*Idem*	94		90	91	91		84
11	"	54		53	61	61		86
3	Basal + cholesterol, 3 g/day	52	54	55	51	51	49	70
5	" " "	76	76	76	76	72	69	78
6	*Idem* + mustard oil	78	78	91	114	126	122	156
7	" "	69	75	90	107	119	128	156
1	" + coconut oil	61	62	90	117	123	126	146
4	" "	65	69	86	101	107	113	135
13	" + sesame oil	87	87	116	197	248	345	491
14	" "	79	78	100	133	167	214	401

TABLE III. Plasma Phospholipids of Monkeys on Different Diets (mg Lecithin/100 ml).

Monkey No.	Treatment	Months after supplements were fed 0	½	1	2	3	4	6
8	Basal diet only	130	135	131	142	132		—
10	*Idem*	136	139	141	137	136		130
11	"	137	132	138	140	140		145
3	Basal + cholesterol	135	142	152	172	185	197	184
5	" "	138	151	165	170	170	178	179
6	*Idem* + mustard oil	138	165	220	262	333	342	334
7	" "	145	153	175	240	286	330	331
1	" + coconut oil	129	132	146	191	284	321	—
4	" "	142	150	161	222	275	318	318
13	" + sesame oil	149	152	156	168	174	178	162
14	" "	137	140	146	159	185	191	186

TABLE IV. Fecal Excretion of Lieberman-Burchard Reacting Sterols by Monkeys on Different Diets (mg/Day).

Monkey No.	Treatment	Months after supplements were fed 0	½	1	2	3	4	6
8	Basal diet only	265		280	282		285	—
10	*Idem*	212		263	271		275	225
11	"	195		281	285		280	215
3	Basal + cholesterol	185	2220	2250	1920	1910	1910	1920
5	" "	282	2590	2650	2350	1820	1880	1800
6	*Idem* + mustard oil	167	2720	2750	2720	1950	1830	752
7	" "	300	2620	2750	2820	1900	1930	861
1	" + coconut oil	309	2890	2860	2690	1880	881	852
4	" "	175	2820	2810	2930	1820	990	960
13	" + sesame oil	111	2790	2750	2420	866	560	396
14	" "	142	2850	2560	2560	899	653	354

the animals fed sesame oil. The rise in plasma ester cholesterol followed patterns similar to total cholesterol.

Plasma phospholipids after cholesterol feeding increased and the increase was considerable after 6 months of cholesterol feeding. The rise in plasma phospholipid was higher in monkeys fed mustard oil and coconut oil and minimum in animals fed sesame oil when fed along with cholesterol.

From the third month fecal excretion of sterols began to diminish in the animals fed cholesterol with the different oil supplements, with maximum decrease in the sixth month in animals of all groups. Animals fed sesame oil showed minimum excretion of fecal sterols. Animals fed cholesterol without oils excreted large quantities of sterols in the feces.

α-Lipoprotein fraction diminished with concomitant increase in percentage of β + O lipoprotein in animals fed cholesterol with mustard oil and sesame oil in the 6th month of experiment. The plasma nonesterified fatty acids increased after feeding cholesterol, and the rise was considerable when sesame oil was fed along with cholesterol.

Discussion. The results show that the degree of unsaturation of the oils has no direct relationship to plasma cholesterol level in rhesus monkeys. Supplementation of cholesterol feeding along with mustard oil (iodine No. 104), sesame oil (iodine No. 110) and coconut oil (iodine No. 9) raised the cholesterol level considerably and fecal excretion of cholesterol diminished proportionately. When the diet was not supplemented with any oil, there were insignificant changes in plasma lipid levels for the first 2 months, but there were marked increases in these values by the sixth month. This is in accord with the observations of Connor *et al.*(2) in man. It has been observed in human experi-

TABLE V. Plasma Lipoproteins and Nonesterified Fatty Acids of Monkeys.

Monkey No.	Treatment	Before supplements: Lipoproteins, % α	Before supplements: Lipoproteins, % β + O	Before supplements: NEFA, μeq/liter	6 mo after supplements: Lipoproteins, % α	6 mo after supplements: Lipoproteins, % β + O	6 mo after supplements: NEFA, μeq/liter
8	Basal diet only	21	79	293	20	80	—
10	*Idem*	22	78	293	19	81	585
11	"	21	79	195	22	78	393
3	Basal + cholesterol	25	75	285	19	81	1050
5	" "	20	80	195	15	85	771
6	*Idem* + mustard oil	19	81	339	10	90	561
7	" "	25	75	339	10	90	561
1	" + coconut oil	22	78	257	23	77	933
4	" "	20	80	212	13	87	1050
13	" + sesame oil	29	71	293	5	95	1755
14	" "	20	80	293	4	96	1989

TABLE VI. Fatty Acid Composition of the Oils Used (Weight %).

Oil	C_6	C_8	C_{10}	C_{12}	C_{14}	C_{16}	C_{18}	C_{20}	C_{22}	C_{24}	Oleic acid	Linoleic acid	Linolenic acid	Erucic acid
Mustard (22)									3.8	1.1	32.3	18.1	2.7	41.5
Coconut (23)	.5	9	6.8	46.4	18	9	1				7.6	1.6		
Sesame (24)						7.8	4.7	.4			49.4	37.7		

ments(16,17) that rapeseed oil and diet containing ethyl linoleate have depressant effect on serum cholesterol level. Both rapeseed oil and mustard oil are rich in erucic acid, whereas sesame oil contains considerable linoleic acid. The depressant effect of the different oils on serum cholesterol level, therefore, may not be due to the fatty acid composition of the oils. It is interesting to note that fecal Lieberman Burchard reacting sterols diminished along with an increase in plasma cholesterol levels. Monkeys fed crystalline cholesterol without the supplement of oils excreted maximum amount of cholesterol in the feces. This suggests that the vegetable oils used in our experiments helped in absorption of cholesterol. The mechanism of action, however, is not clear.

In monkeys fed cholesterol with sesame oil, although the plasma cholesterol was highest, the phospholipid content of plasma was low as compared to the other groups of animals. The altered cholesterol/phospholipid ratio in monkeys fed sesame oil indicates possibly that fatty acids esterify cholesterol to a greater degree than they do phospholipid since the ester cholesterol of plasma was highest in animals fed sesame oil.

A decrease in α-lipoprotein % and an increase in the ratio of β + O to α-lipoprotein were observed in monkeys fed the different oils with the exception of one of the monkeys fed coconut oil. Similar alteration in ratio was reported in patients with atherosclerosis (18). It is, however, not known if the animals have developed atherosclerosis as the arterial studies have not yet been made.

It is difficult to account for the increased plasma nonesterified fatty acid (NEFA) levels in monkeys fed cholesterol with or without the different oils. Plasma NEFA is directly related to the plasma level of glucose (19). Popjak reported that addition of cholesterol to the diet increased mobilization of fat(20). It has been suggested(21) that the clearance of fat from blood is dependent on normal carbohydrate utilization. It may be possible that the increase in plasma NEFA values is due to defective utilization of carbohydrates in these animals.

Summary. Rhesus moneys were fed cholesterol along with mustard oil (iodine No. 104), coconut oil (iodine No. 9), and sesame oil (iodine No. 110). Fecal cholesterol and plasma cholesterol, phospholipid, lipoproteins and nonesterified fatty acids (NEFA) were estimated at intervals up to 6 months. Supplementation of cholesterol feeding with mustard and coconut oils raised plasma cholesterol and reduced fecal cholesterol to the same degree. The above changes were most marked when the animals were fed sesame oil. A relative decrease in the α-lipoprotein % to β + O lipoproteins was observed in monkeys fed the different oils. Plasma NEFA increased after cholesterol feeding and was highest in animals fed sesame oil. The depressant effect of the different oils on plasma cholesterol level does not seem to be due to saturation, unsaturation or fatty acid composition of the oil.

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Intracellular Sodium Components in Rat Skeletal Muscle.* (27189)

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Glycerated frog skeletal muscle was found by Fenn(1) to contain sodium after immersion in 50% glycerol for times varying from 12 hr to 4 days. The temperature is not stated. The quantity present was 3.82 to 12.3 meq/kg wet weight. The amount of sodium did not appear to be related to the time of immersion in glycerol. More recently Craig(2) found that the sodium content of frog skeletal muscle decreased steadily after being placed in glycerol, from time zero when sodium value was 27.4 ± 0.3‡ meq/kg, to

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‡ Standard deviation of mean.